Dehydrin Expression and Drought Tolerance in Seven Wheat Cultivars

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ABSTRACT

The winter wheat (*Triticum aestivum* L.) producing region of the U.S. Pacific Northwest (PNW) is subject to periods of water deficit during sowing and grain filling. Improving the genetic adaptation of wheat to drought stress represents one of the main objectives of regional breeding programs. One biochemical response to dehydrative stress is the accumulation of a family of proteins called dehydrins, which are believed to protect membranes and macromolecules against denaturation. Although previous studies demonstrated the accumulation of dehydrins in drought-stressed wheat, little was known about the relation of dehydrin expression to acquisition of drought tolerance in specific varieties adapted to the PNW. We characterized dehydrin accumulation during the exposure of seven cultivars ('Connie', 'Gene', 'TAM105', 'Rod', 'Hiller', 'Rhode', and 'Stephens') to progressive drought stress in four separate experiments. The objective was to identify differences in the nature or timing of dehydrin expression in these cultivars and to learn whether dehydrin expression was associated with the acquisition of stress tolerance during seedling development. Expression of a 24-kDa dehydrin was observed in Connie, TAM105, and Gene after 4 d of stress and at subsequent sampling dates while no dehydrins were detected in nonstress control plants. Dehydrin expression was significantly delayed in the remaining cultivars. The presence of this dehydrin was related to acquisition of drought tolerance characterized by a greater maintenance of shoot dry matter production in Connie, TAM105, and Gene. Although the role of these proteins remains unknown, their association with stress tolerance suggests that dehydrins might be used to improve the adaptation to drought.

Most wheat-producing regions of the world are subject to water deficits during some part of the growing season (Moustafa et al., 1996). The impacts of these water deficits on grain development and yield depend on their severity and the stage of plant growth during which they occur. Seedling emergence is one stage of growth that is sensitive to water deficit. In Mediterranean environments like the PNW, dry conditions during emergence and early growth along with low temperatures during winter and high temperatures and increasing water demands at the end of spring, result in low yields because of the inability of plants to produce adequate dry matter (Regan et al., 1992). Many producing regions of the world, including the PNW are subjected to water deficits during the seedling stage since winter wheat is sown during autumn into dry soil and rarely receives additional moisture during emergence. The lack of precipitation during seedling emergence represents a major cropping risk to producers. Consequently, there is need to improve the genetic tolerance of wheat to drought at the seedling stage.

Plant breeding efforts to improve drought tolerance would be aided by the identification of biochemical markers associated with improved field performance under drought conditions. Dehydrins, also known as late embryogenesis abundant (LEA) D11 (Dure, 1993) proteins represent potential markers. Dehydrins are members of a family of proteins that are expressed after plants are exposed to stresses with a dehydrative component. This family of proteins is characterized by the presence of a consensus amino acid sequence (EKK GIMDKIKELPG) near the carboxy terminus (Close et al., 1993). Dehydrins can be detected by means of antibodies prepared against this consensus sequence (Close et al., 1993) and have been identified in at least 30 diverse plant species including wheat (Campbell and Close, 1997).

An association between tolerance to stresses with a dehydrative component (drought, freezing, or salinity) and the expression of dehydrin proteins has been observed in some crop species. Houde et al. (1992) found that the expression of a specific dehydrin (WSC120) accompanied the development of freezing tolerance in eight species of Gramineae. Tolerance to chilling temperatures during emergence was correlated with the expression of a 35-kDa dehydrin in two genetically similar cowpea [*Vigna ungiculata* (L.) Walp] sublines that differed in their expression of this dehydrin (Ismail et al., 1997). Lim et al. (1999) also found a positive association between cold hardiness and a dehydrin protein in *Rhododendron*. Danyluk et al. (1998) showed that the WCOR410 dehydrin protein accumulated near the plasma membrane during cold acclimation of wheat and suggested that this accumulation protected the integrity of the plasma membrane when plants were subjected to stress. Zhu et al. (2000) reported increased expression of dehydrin genes during the development of freezing tolerance in a more tolerant barley (*Hordeum vulgare* L.) cultivar Dicktoo relative to that which occurred in 'Morex', a less tolerant variety. Cellier et al. (1998), using two sunflower (*Helianthus annuus* L.) inbred lines, one tolerant and one susceptible to drought stress, showed a higher accumulation of mRNA transcripts corresponding to HaDhn1 and HaDhn2 genes in the tolerant line that was associated with cellular turgor maintenance under drought stress.

Although genotypic differences in dehydrin expression have been demonstrated in responses to cold and drought tolerance, it is important to study the expression of dehydrins in relation to changes in leaf water potential when seedlings are exposed to drought. The purpose

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of this research was to evaluate the association between dehydrin proteins and drought stress tolerance during the seedling stage by monitoring seedling dehydrin expression in seven wheat cultivars subjected to controlled drought conditions in greenhouse experiments.

MATERIALS AND METHODS

Plant Material

Seven winter wheats including Stephens, Gene, Rod, Hiller, Rhode, Connie, and TAM 105, one winter barley cultivar, 'Strider', and 'Celia', a winter triticale (× *Triticosecale* Wittmack), were evaluated in two greenhouse experiments under stressed (drought) and nonstressed conditions. Stephens, Gene, and Rod are common soft white genotypes, Hiller, and Rhode are soft white club wheats, Connie is a durum genotype, and TAM 105 is a hard red wheat. Cultivars were selected to represent genotypes adapted to the Pacific Northwest of the USA, with the exception of TAM 105, a cultivar with known drought tolerance, adapted to the central Great Plains of the USA (Winter et al., 1988).

Plant Growth and Trial Development

Growth conditions and trial development were the same for both experiments. A system similar to the one developed by Snow and Tingey (1985) was used to impose drought stress. Pots with sterilized sand were placed on top of cylinders containing florist foam blocks as a hydraulic conducting medium. Roots were prevented from growing down the florist foam blocks by means of a 5-μm nylon mesh at the bottom of the pot. The cylinders were connected to a tank (one for the stress treatment and one for the nonstressed control) containing a complete nutrient solution. Seeds of each cultivar were germinated at 20°C for 48 h and, seedlings were selected for size and vigor. Ten seedlings of each cultivar were planted in a row with each pot containing three cultivars. Pots were placed in containers with a complete nutrient solution for 15 d (when the seedlings had approximately three leaves) and then transferred to the cylinders when the experiment started (first day). By using a floating valve in each tank, the water level was maintained at 4 cm from the bottom of the pots in the nonstressed treatment and at 12 cm in the stressed treatment (Saulescu et al., 1995). To increase the stress intensity, a ceramic disk (Soil Moisture Equipment Corp., Santa Barbara, CA) with an air exclusion of 0.5 MPa was inserted between the base of the pot and the florist foam in the stress treatment. The experimental design was a split-plot with four replications. Stress levels (drought and well-watered conditions) were the main plots, and cultivar subplots were arranged in a randomized complete block design. From the first to the fifth day of treatment, leaf water potential (ψ_l) was measured daily by means of a pressure chamber. Leaf water potential was measured for each cultivar and stress treatment combination on one of the last fully expanded leaves in three replications. All ψ_l measurements were made between 1200 and 1400 h. Immediately after ψ_l was recorded, one seedling of each plot was cut and placed on dry ice. These are Samples 1 through 5 in this study. When all the determinations in the experiment were finished, the samples were stored at −80°C. After five days of treatment, plants were allowed to grow for an additional week to assure a measurable difference in the shoot dry matter accumulation between stress and nonstressed treatments. At that time, the remaining plants were cut to the soil level and stored at −80°C.

Western Blots

Only wheat cultivars were analyzed for the presence of dehydrin proteins. All sampled seedlings were lyophilized and total dry weight per plot was recorded. Tissue samples were ground to a powder in liquid nitrogen with a mortar and a pestle. Stress and nonstress samples within a sampling date were bulked to make one sample, giving 14 samples for analysis per sampling date. Protein was extracted by grinding the powdered tissue in the presence of E buffer [125 m*M* Tris-HCL pH 8.8, 1% (w/v) SDS, 10% (v/v) glycerol, 50 m*M* $Na_2S_2O_2$] according to Martinez-Garcia et al. (1999) until a homogeneous mixture was obtained. The extract was transferred to a 1.5-mL microfuge tube and centrifuged at 14 000 g for 6 min. An aliquot of the supernatant was used for protein concentration determinations and the rest was diluted (1/10 of the volume) with Z buffer [125 m*M* Tris-HCL pH 6.8, 12% (w/v) SDS, 10% (v/v) glycerol, 22% (v/v) β-mercaptoethanol, 0.001% (w/v) bromophenol blue] (Martinez-Garcia et al., 1999). The total protein concentration of each sample was determined by the Bio-Rad Protein Assay (Bio-Rad Laboratories, Hercules, CA). Samples containing 10 μg of total protein extracted from the seven cultivars (stressed and nonstressed) plus a prestained molecular weight marker (BenchMark, GIBCO-BRL, Grand Island, NY) were electrophoresed in SDS-PAGE gels [14% (w/v) acrylamide] by means of Mini Protean II cells (Bio-Rad), and then transferred to PVDF membranes using Mini Trans-Blot cells (Bio-Rad). A positive dehydrin control consisting of protein extracted from Gene under drought stress was used in western blots corresponding to the first and the sixth sampling dates. The membranes were blocked in 5% (w/v) nonfat dried milk in phosphate buffered saline for 18 h at 4°C. Transferred proteins were probed with a primary dehydrin antibody (StressGen Biotechnologies Corp, Victoria, Canada) prepared against a synthetic peptide containing the conserved sequence EKKGIMDKIKELPG (Close et al., 1993). Reactive bands were detected with an anti-rabbit IgG secondary antibody conjugated to horse radish peroxidase (Immunopure, Pierce, Rockford, IL) by a chemoluminescent substrate (SuperSignal West Pico for HRP, Pierce, Rockford, IL) and clear blue X-ray film (CL-XPosure, Pierce, Rockford, IL).

Statistical Analysis

Data analysis was performed by analysis of variance using GLM procedure (SAS Institute, Cary, NC) on dry weights. To assess the level of drought tolerance of each cultivar on the studied traits, the drought susceptibility index (S) (Fischer and Maurer, 1978) was calculated as

$$S = (1 - Y_D/Y_1)/(1 - Y_{MD}/Y_{MI}). \quad [1]$$

Where Y_D is the plot value for a genotype under stress, Y_I is the plot value for the same genotype under nonstress, and Y_{MD} and Y_{MI} are the mean value of the experiment under stress and nonstress conditions, respectively. The rate of decrease in leaf water potential per day of stress was estimated as the slope of the linear regression of ψ_l on days of stress.

RESULTS AND DISCUSSION

Significant differences were observed between treatments (drought and well-watered plots) for shoot dry matter accumulation in the first ($P < 0.01$) and second ($P < 0.01$) experiments, indicating the effectiveness of

Table 1. Mean squares from the analysis of variance in shoot dry weight of seven wheat cultivars in two independent experiments.

Source	df	Mean Squares	
		Shoot Dry Weight (g)	
		First Experiment	Second Experiment
Rep	3	3.72*	2.17*
Stress	1	46.23**	43.08**
Rep × Stress	3	0.57 ns	0.81 ns
Cultivar (C)	6	1.84 ns	2.06*
C × Stress	6	0.57 ns	0.66 ns
Residual	36	0.93	0.70

*, Significant at 0.05 probability level.
**, Significant 0.01 probability level.
ns = nonsignificant.

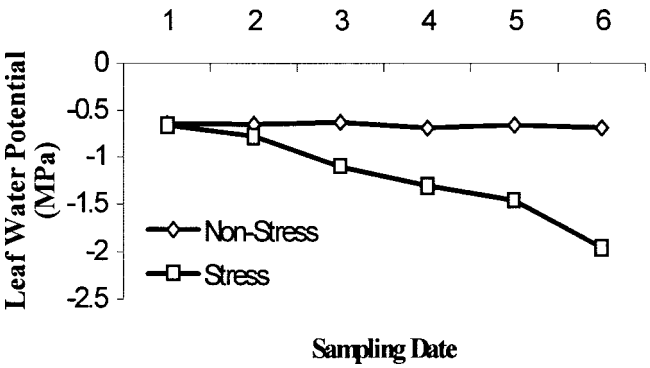

Fig. 1. Leaf water potential (MPa) in control and drought-treated wheat. Values are averages calculated from measurements of seven wheat cultivars in two independent experiments. Measurements were made on sampling dates 1 through 6 which represented seedlings at 16, 17, 18, 19, 20, and 25 d of age, respectively.

the treatment to impose stress (Table 1). The average reduction in dry matter accumulation of drought-treated plants (data not shown) with respect to the well-irrigated controls was 35.2% (5.14 g vs. 3.33 g, respectively) in the first experiment and 37.5% (4.73 g vs. 2.98 g, respectively) in the second experiment.

In the well-watered treatment, average ψ_l (over all cultivars in Exp. 1 and 2) remained high during the experiment (from −0.66 MPa to −0.71Mpa). In contrast, the stress treatments showed a progressive decrease in the average ψ_l from −0.66 MPa in the first sampling date to −1.96 MPa in the sixth sampling date (Table 2 and Fig. 1). However, there were no significant differences among cultivars (Table 3) in the rate of reduction of ψ_l per day of stress ($RD\psi_l$).

Dehydrin Accumulation and Stress Tolerance

Dehydrin accumulation was characterized during exposure of the seven cultivars to progressive drought stress. Dehydrins are usually expressed in cereal seedlings during gradual exposure to dehydrative stress (Close and Chandler, 1990). By the first sampling date (0 d of stress, Fig. 2A) the ψ_l in stress and nonstress treatments were similar (−0.66 MPa vs. −0.64 MPa, respectively, Table 2), as expected, and no dehydrins were detected. Subsequently, drought stress progressed as indicated by the ψ_l value of −1.10 MPa at the third sampling date. However, no dehydrin bands were observed in any cultivar at this point (western not shown).

On the fourth sampling date (4 d of stress, Fig. 2B), when average ψ_l in the stressed plants was measured at −1.31 MPa, a dehydrin of 24 kDa was detected in cultivars Connie, TAM105, and Gene. The latter genotype also showed a minor expression of a 19-kDa protein that reacted with the antidehydrin antibody. No dehydrins were observed in the other cultivars under stress nor were any seen in any cultivars in the nonstress treatment. At the fifth sampling date (ψ_l = −1.47 MPa in stress treatment) the 24-kDa dehydrin and faint bands between 14 and 19 kDa were present (Fig. 2C) in the same three cultivars. No dehydrins were observed in any of the well-watered plants or other cultivar subjected to drought. Close and Chandler (1990) also detected a 25-kDa dehydrin in stressed wheat and barley seedlings along with faint bands between 18 and 21 kDa and no dehydrin proteins in well-watered plants. Following the reasoning of Close et al. (1993), these faint bands may be intact proteins or degradation products.

On the sixth sampling date, after seedlings had been subject to 12 d of progressive stress and the average ψ_l was reduced to −1.96 MPa in stressed plants, dehydrin

Table 2. Mean leaf water potentials of seven wheat cultivars for each sampling date in two independent experiments and mean rate of decrease in leaf water potential ($RD\psi_l$) in drought-stressed plants.

Cultivar	Stress Level	ψ_l (MPa) in Sampling Dates						$RD\psi_l$
		First	Second	Third	Fourth	Fifth	Sixth	(MPa day^{-1})
Gene	NS†	−0.61	−0.63	−0.65	−0.69	−0.66	−0.67	–
Gene	S†	−0.62	−0.79	−1.10	−1.27	−1.49	−1.90	−0.24
Rod	NS	−0.66	−0.68	−0.65	−0.71	−0.69	−0.71	–
Rod	S	−0.62	−0.91	−1.14	−1.40	−1.41	−1.81	−0.23
Stephens	NS	−0.65	−0.65	−0.61	−0.67	−0.63	−0.68	–
Stephens	S	−0.68	−0.78	−1.14	−1.25	−1.50	−2.00	−0.22
Rhode	NS	−0.62	−0.58	−0.63	−0.70	−0.62	−0.63	–
Rhode	S	−0.67	−0.74	−1.08	−1.19	−1.50	−1.85	−0.24
Connie	NS	−0.61	−0.62	−0.58	−0.64	−0.65	−0.71	–
Connie	S	−0.66	−0.76	−1.11	−1.24	−1.47	−1.91	−0.25
TAM105	NS	−0.62	−0.66	−0.60	−0.70	−0.68	−0.72	–
TAM105	S	−0.58	−0.72	−1.07	−1.22	−1.35	−1.78	−0.25
Hiller	NS	−0.66	−0.65	−0.63	−0.68	−0.65	−0.67	–
Hiller	S	−0.65	−0.71	−1.06	−1.36	−1.40	−1.92	−0.25
Average NS		−0.64	−0.65	−0.63	−0.69	−0.66	−0.69	
Average S		−0.66	−0.78	−1.10	−1.31	−1.47	−1.96	

† Nonstress.
† Stress.

Table 3. Combined analysis of variance for drought susceptibility index of shoot dry matter production (S_{DM}), and rate of decrease in leaf water potential ($RD\psi_l$) in seven wheat cultivars in two independent experiments.

Source	Df	Mean Squares	
		S_{DM}	$RD\psi_l$ (MPa day^{-1})
Exp.	1	0.0321 ns	0.0030**
Rep (Exp)	6	0.1856 ns	0.0007 ns
Cultivar (C)	6	0.3019**	0.0008 ns
High vs. Low	1	0.9975**	0.0001 ns
C × Exp.	6	0.2022*	0.0004 ns
Residual	36	0.0790	0.0003

* Significant at 0.05 probability level.
** Significant 0.01 probability level.
ns = nonsignificant.

proteins were detected in Hiller (24 kDa), Stephens (19 kDa), and Rhode (19 kDa) (Fig. 2D). Since no samples were collected between Days 6 and 11, it cannot be precisely established in which day the production of dehydrins was induced in those cultivars. Nevertheless, Connie, TAM105, and Gene produced dehydrins at least 2 d earlier. No significant differences among the seven cultivars were observed in $RD\psi_l$ indicating that the imposed water stress was similar along the experiments for all the cultivars studied. In spite of the common stress conditions, Connie, TAM105, and Gene accumulated dehydrins at a higher ψ_l (−1.24 MPa, −1.22 MPa, and −1.27 MPa, respectively, at the fourth sampling date) than the rest of the cultivars that showed dehydrins after the fifth sampling date with average ψ_l between −1.47 MPa and −1.96 MPa. This differential dehydrin accumulation can result from differences in gene regulation or in genome organization such as a higher number of dehydrin gene copies (Labhilili et al., 1995).

The drought susceptibility index (S) (Fischer and Maurer, 1978) was calculated to determine the extent of drought tolerance or susceptibility of each cultivar. This index measures the ratio of the stress to well-irrigated plot values of a trait for each genotype in relation to the same ratio for the mean of all genotypes in the experiment (Clarke et al., 1992). As a consequence, two genotypes with similar proportional reduction from stress to well-watered conditions will show a similar S value, even if one is a high yielding genotype and the other a low yielding genotype. The combined analysis of variance (Table 3) showed significant differences between cultivars for the shoot dry matter susceptibility index (S_{DM}) ($P < 0.01$). To determine whether the observed differential dehydrin expression was related to drought stress tolerance, an orthogonal contrast between early (Connie, TAM105, and Gene) and late (Rod, Rhode, Stephens, and Hiller) dehydrin induction was performed for S_{DM}. The contrast revealed significant differences for S_{DM} ($P < 0.01$), with a lower mean for the early production group (0.79 vs. 1.06) (Table 3). Cultivars showing lower S values are more tolerant to

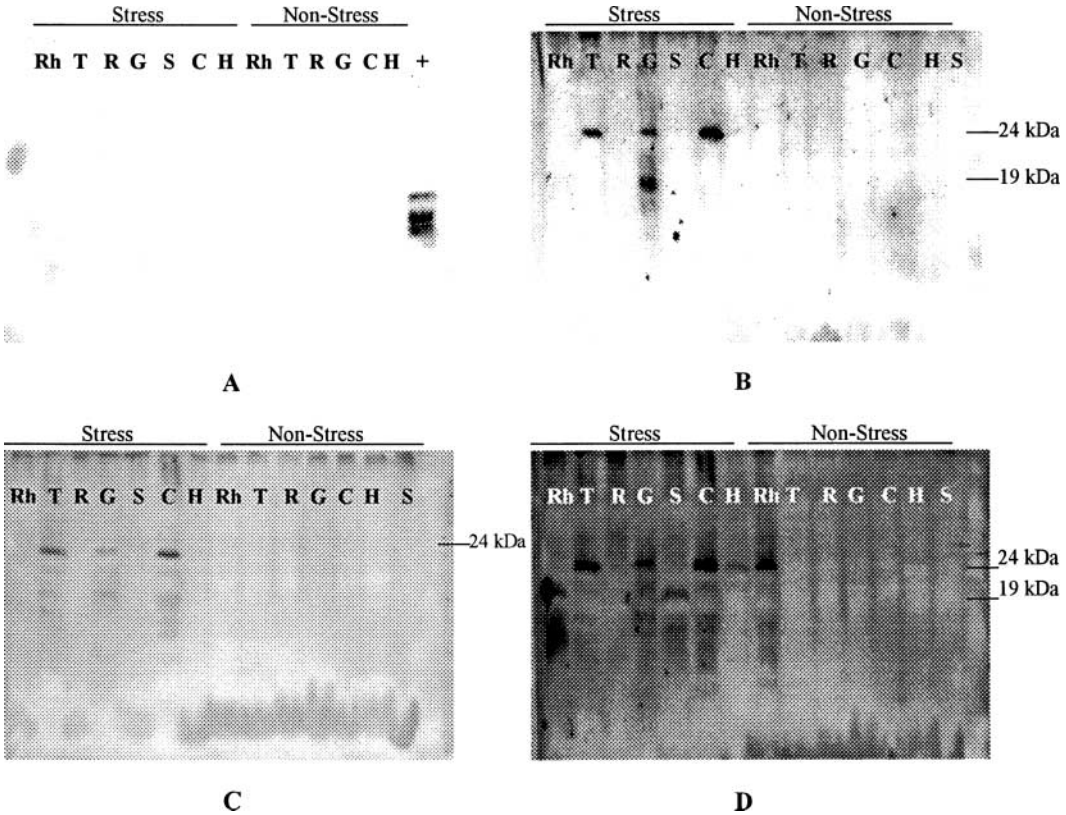

Fig. 2. Expression of dehydrins in wheat leaves collected after 0 (A), 4 (B), 6 (C), and 12 (D) days of progressive drought stress in seedlings of cultivars Connie (C), Rhode (Rh), TAM105 (T), Hiller (H), Gene (G), Stephens (S), and Rod (R). Nonstress treatments consisted of well-watered plants treated identically to stressed plants in all other aspects. + = positive control (protein extract from Gene wheat subject to drought stress, used only on westerns A and D). Dehydrin proteins were detected using a commercial anti-dehydrin antibody prepared against the consensus dehydrin amino acid sequence EKKGIMDKIKELPG (Close et al., 1993).

stress, since they have a lower reduction in the value of a trait from nonstress to stress conditions relative to the overall reduction observed for all cultivars (see Eq. [1]). Consequently, the significantly lower mean S_{DM} indicates a positive association between the presence of dehydrin proteins and drought tolerance. On the basis of the LSD test (Table 4), Connie, TAM105, and Gene showed the lowest values for S_{DM} together with Hiller, a cultivar that represents an exception since it showed a later induction. The mechanisms that conferred drought tolerance to Hiller apparently were not related to early dehydrin expression but crossing this cultivar with another soft white genotype like Gene might be an effective means to combine early dehydrin accumulation with other stress tolerance mechanisms.

Dehydrins were associated with drought stress tolerance (Labhilili et al., 1995; Bettey et al., 1998; Cellier et al., 1998; Giordani et al., 1999), freezing tolerance (Houde et al., 1992; Ismail et al., 1997; Lim et al., 1999; Zhu et al., 2000), and salt tolerance (Galvez et al., 1993) in different plant species including wheat. On the basis of their physical properties, a role in stabilizing membranes and macromolecules in the cytoplasm is proposed (Campbell and Close, 1997). An interaction between dehydrins and membranes was suggested in *Arabidopsis* by in vitro studies of freezing tolerance (Tomashow et al., 1996). Moreover, Danyluk et al. (1998) provided evidence for the accumulation of the WCOR410 dehydrin protein near the plasma membrane during cold acclimation of wheat, and suggested a protective role of the plasma membrane in plants subjected to stress. Although, the specific role of dehydrins remains unknown (Campbell and Close, 1997), as more dehydrin genes are mapped and sequenced, and their expression pattern studied, the opportunities for the use of dehydrins in improving the genetic adaptation to drought stress will increase.

In our study, the presence of dehydrin proteins in seedling leaves under increasing drought stress conditions was correlated to a reduced impact of drought stress on shoot dry matter production in the most tolerant cultivars Connie, TAM105, and Gene. Stress tolerance during the seedling stage is very important in areas of the PNW with low annual rainfall (around 300 mm), where stress can reduce seedling growth (Donaldson, 1996). Tolerant cultivars should be able to grow more vigorously, cover more ground area, and reduce the loss of available water in the soil by evaporation. Although early vigor may be a disadvantage if too much water is extracted early from the soil, it is usually considered an advantage in wheat for yield (Turner and Nicholas, 1987; Slafer and Araus, 1998).

Dehydrins have been associated with cold tolerance in many plant species, a desirable characteristic for cultivars adapted to the PNW where low temperatures during winter can produce crop damage. However, two of the cultivars showing early dehydrin production (Gene and Connie) were less winter hardy than the other cultivars used in this study (unpublished data). It is possible that expression of other dehydrin genes is involved in cold tolerance.

Table 4. Mean drought susceptibility index for shoot dry matter production in wheat cultivars based on two independent experiments.

Cultivar	Shoot Dry Matter Drought Susceptibility Index (S_{DM})
Hiller	0.82
Rhode	1.13
Stephens	1.08
Rod	1.23
TAM105	0.72
Gene	0.83
Connie	0.83
LSD (0.05)	0.28

In a separate study, the same seven cultivars were evaluated in two greenhouse experiments where plants were subjected to drought stress at grain filling. In that study, Connie, TAM105, and Gene also were significantly more drought tolerant, showing increased expression of a 24-kDa dehydrin and less reduction in ψ_l per day of stress relative to the remaining cultivars. The correlation between our results in seedlings and adult plants opens the possibility for the use of this dehydrin to develop a screening technique to select for drought stress tolerance, but the results must be confirmed. One approach to confirm these results is to develop selection experiments. A segregating population can be formed by crossing two cultivars contrasting in drought stress tolerance and dehydrin expression (i.e., Gene and Rhode) to test if the relation of dehydrin accumulation with drought tolerance can be used to select for more tolerant genotypes.

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